Topical Review

Evidence for altered placental blood flow and vascularity in compromised pregnancies

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The placenta is the organ that transports nutrients, respiratory gases, and wastes between the maternal and fetal systems. Consequently, placental blood flow and vascular development are essential components of normal placental function and are critical to fetal growth and development. Normal fetal growth and development are important to ensure optimum health of offspring throughout their subsequent life course. In numerous sheep models of compromised pregnancy, in which fetal or placental growth, or both, are impaired, utero-placental blood flows are reduced. In the models that have been evaluated, placental vascular development also is altered. Recent studies found that treatments designed to increase placental blood flow can 'rescue' fetal growth that was reduced due to low maternal dietary intake. Placental blood flow and vascular development are thus potential therapeutic targets in compromised pregnancies.

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> *'During pregnancy the blood flow to the uterus increases to satisfy the metabolic demands of the conceptus.'* Giacomo Meschia (1983)

The importance of the placental circulation to fetal growth has been recognized since ancient times. For example, in his great treatise, On the Generation of Animals (*ca* 340 B.C.), Aristotle stated:

> *'The [umbilical] vessels join on the uterus like the roots of plants and through them the embryo receives its nourishment.'*

In this brief review, we will evaluate the importance of the placental circulation to fetal growth and development, and then will attempt to answer the question, 'Is placental vascular growth or function altered in compromised pregnancies?' We will define compromised pregnancies broadly as those in which either fetal or placental growth, or both, are reduced. Once the evidence has been reviewed, we will ask the clinically relevant question, 'Can placental blood flow or vascularity be used as a therapeutic target to 'rescue' fetal or placental growth in compromised pregnancies?' Although we will concentrate on data in ruminants, for which there are numerous well-established models of compromised pregnancy, where applicable, data from other species will be discussed.

The importance of placental blood flow and vascularity to normal fetal growth and development

The placenta's primary role is to provide for physiological exchange between the fetal and maternal systems (Meschia, 1983; Reynolds & Redmer, 1995). In this context, the importance of the placental circulation to successful pregnancy is exemplified by the close relationships among fetal weight, placental size, and uterine and umbilical blood flows during normal pregnancies in many mammalian species (Reynolds *et al.* 2005*a*,*b*).

Uterine and umbilical blood flows, which primarily represent the circulation to the maternal and fetal portions of the placenta, respectively (Ramsey, 1982; Mossman, 1987), increase exponentially throughout gestation, essentially keeping pace with fetal growth (Reynolds & Redmer, 1995; Magness, 1998). For example, in sheep the absolute rate of uterine blood flow increases by approximately 3-fold (0.4–1.2 l min^{-1}) throughout the last half of pregnancy (D 71–131, or 49–90% of gestation, respectively; Meschia, 1983). Over a similar interval of gestation, uterine blood of cows increases by 4.5-fold (2.9–13.2 l min^{-1}; Reynolds *et al.* 1986) and that of humans increases by 2.5-fold (0.33–0.83 l min^{-1};

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Konje *et al.* 2003). The continual increase in the rate of uterine blood flow also seems to be the case for the other mammalian species (Meschia, 1983; Metcalfe *et al.* 1988; Reynolds & Redmer, 1995). Similarly, in sheep and cows, umbilical (fetal placental) blood flow increases dramatically throughout pregnancy, such that it keeps pace with fetal growth throughout the last half of gestation (Rudolph & Heymann, 1970; Bell *et al.* 1986; Reynolds *et al.* 1986; Reynolds & Ferrell, 1987; Molina *et al.* 1991). Although not measured directly, umbilical blood flow velocity increases and resistance decreases throughout the last half of gestation in humans (Gadelha Da Costa *et al.* 2005). Not only do absolute utero-placental flows increase throughout pregnancy, but importantly, the proportion of the total uterine and umbilical blood flows received by the caruncular and cotyledonary tissues, respectively, also increases as gestation progresses (Makowski *et al.* 1968*a*,*b*; Rosenfeld *et al.* 1974; Meschia, 1983).

Other critical placental functions such as the transport of oxygen and water also keep pace with fetal growth (Barcroft, 1946; Faber & Thornburg, 1983; Meschia, 1983; Reynolds & Redmer, 1995). As reported for umbilical blood flow, oxygen uptake and water transport remain constant when expressed per unit of fetal weight (Meschia, 1983; Reynolds *et al.* 1986; Reynolds & Ferrell, 1987). Similarly, fetal uptake of glucose essentially keeps pace with the rate of fetal growth (Reynolds *et al.* 1986; Molina *et al.* 1991).

Placental transport capacity may increase as gestation advances due to an increase in the rate of extraction of substances from uterine or umbilical blood (Barcroft, 1946; Faber & Thornburg, 1983; Meschia, 1983). In fact, extraction of oxygen per unit of uterine blood increases from mid- to late gestation in sheep and cattle (Meschia, 1983; Reynolds *et al.* 1986). Placental uptake is calculated based on the Fick principle:

$$\text{Uptake} = \text{blood flow}[\text{A} - \text{V}],$$

where [A − V] represents the arterio-venous concentration difference. Thus, transplacental exchange could increase by increasing the rate of extraction (the A − V concentration difference) or by increasing the rate of blood flow, or both.

Based on numerous studies, it seems that increased blood flow is an important, if not the primary, mechanism of increased transplacental exchange throughout gestation (Meschia, 1983; Reynolds *et al.* 1986; Metcalfe *et al.* 1988; Ferrell, 1989). For example, in cattle oxygen extraction by the gravid uterus increases only 0.4-fold, whereas uterine blood flow increases approximately 4.5-fold from mid- to late gestation. Thus, increased uterine blood flow accounts for most of the 5- to 6-fold increase in total gravid uterine oxygen uptake. The 16-fold increase in oxygen uptake of the bovine fetus from mid- to late gestation also can be accounted for primarily by the increased rate of umbilical blood flow (Reynolds *et al.* 1986). Similarly in sheep, gravid uterine oxygen extraction increases approximately 0.4-fold from mid- to late gestation, whereas uterine blood flow increases approximately 3-fold (Meschia, 1983). Furthermore, the large increases in gravid uterine and fetal uptakes of glucose, lactate, and amino acid nitrogen from mid- to late gestation in cattle seem to depend primarily on large increases in uterine and umbilical blood flows because the arterio-venous concentration differences for these nutrients remain relatively constant (Reynolds *et al.* 1986; Reynolds & Redmer, 1995).

Thus, adequate blood flow to the placenta seems to be critical for normal fetal growth. At least for those substances that are diffusion limited, such as glucose, increased abundance of specific transporters and an increase in the maternal to fetal concentration gradient also seem to be important components of increased transplacental exchange (Bell *et al.* 1999). Nevertheless, gravid uterine and umbilical glucose uptakes, which provide for about 60% of fetal metabolic needs (Reynolds *et al.* 1986; Bell *et al.* 1999), are reduced approximately in proportion to the reduction in placental mass and blood flows in pregnancies compromised nutritionally or by environmental heat stress (Reynolds *et al.* 1985; Thureen *et al.* 1992; Wallace *et al.* 2002, 2005).

Based on the concept that chronic increases in blood flow to any growing tissue depend on vascular growth, or angiogenesis, Meschia (1983) reasoned that 'the large increase of blood flow to the uterus during pregnancy ... results primarily from the formation and growth of the placental vascular bed.' In fact, numerous studies have indicated that angiogenesis is indeed a major component of the increase in placental blood flow throughout gestation, and establishment of functional fetal and placental circulations is one of the earliest events during embryonic/placental development (Reynolds & Redmer, 1995; Magness, 1998; Charnock-Jones *et al.* 2004; Kaufmann *et al.* 2004; Mayhew *et al.* 2004; Reynolds *et al.* 2005*a*,*b*).

Evidence that placental blood flow and vascularity are altered in compromised pregnancies

Since adequate blood flow to the placenta is critical for normal fetal growth, it is not surprising that conditions associated with reduced rates of fetal and placental growth (e.g. maternal or fetal genotype, increased numbers of fetuses, maternal nutrient deprivation or excess, environmental heat stress, high altitude) are associated with reduced rates of placental blood flow and reduced fetal oxygen and nutrient uptakes in numerous mammalian species, including humans (reviewed in Reynolds & Redmer, 1995; Poston, 1997; Mayhew *et al.* 2004; Reynolds *et al.* 2005*a*; Luther *et al.* 2005; Wallace *et al.* 2005). In

Table 1. Changes in fetal and placental weights, uterine and umbilical blood flows and placental vascularity in various models of compromised pregnancy in sheep

Model	Day of gestation	Fetal wt	Placental wt	Uterine blood flow	Umbilical blood flow	Vascularity	Footnote
Overfed adolescent	130–134	↓20–28%	↓45%	↓36%	↓37%	↓31% (total capillary vol.)	1
Underfed adolescent	130	↓17%	NSE*	—	—	↓20% (cap. area density, CAR)	2
Underfed adult	130–144	↓12%	–	↓17–32%	NSE	↓14% (cap. area density, CAR)	3
Adolescent *versus* adult	135	↓11%	↓29%	—	—	—	4
Genotype	130	↓43%	↓47%	—	—	↑36%	5
Heat-stressed adult	133–135	↓42%	↓51%	↓26%	↓60%	—	6
Multiple pregnancy	140	↓30%	↓37%	↓23%	—	↓30% (total cap. vol., COT)	7
High dietary Se	135	NSE	↓24%	—	—	↑20% (cap number density, COT)	8
Hypoxic (hypobaric) stress	140	NSE	—	↓35%	—	↑ (cap. area density, CAR & COT)	9

[1]Wallace *et al.* (2002); Redmer *et al.* (2004*a*). Uterine blood flow measured on day 130, fetal and placental weights on day 134 of gestation.

[2]Luther *et al.* (2005). Although capillary area density (capillary area as a per cent of tissue area) was reduced by 20% ($P < 0.001$) in maternal caruncle, capillary number density (capillary number per unit tissue area) was increased by 23% ($P < 0.09$).

[3]Chandler *et al.* (1985); Leury *et al.* (1990); Newnham *et al.* (1991); Kelly, 1992); Arnold *et al.* (2001). When nutrient restriction was severe (30–40% of full-fed controls) and during late pregnancy (day 120–144), uterine blood flow was reduced by 20–33%; when nutrient restriction was during mid-pregnancy, uterine blood flow was reduced by 17% or unaffected; in addition, capillary area density only tended ($P < 0.09$) to be reduced at day 130.

[4]Borowicz *et al.* (2005*a*). Adolescents were peri-pubertal (approx. 7 months of age) and adults were approx. 1 year and 7 months of age; data are summarized for both Romanov (small-framed and small birth weight) and Columbia (large-framed and large birth weight) breeds.

[5]Scheaffer *et al.* (2004); Borowicz *et al.* (2005*a*). Comparison for adult pregnancies in Romanov *versus* Columbia breeds. Although individual fetal weights were reduced by 43%, total fetal weight was similar or greater in Romanov *versus* Columbia ewes because of the larger number of fetuses in Romanovs. compared with Columbias (3–4 *versus* 1–2).

[6]Regnault *et al.* (2003). Adult ewes were heat stressed from day 80–120 of gestation.

[7]Data were expressed as per fetus for single- *versus* triplet-bearing ewes (fetal and placental weights, and placental vascularity; Grazul-Bilska *et al.* 2006), or for single- *versus* twin-bearing ewes (uterine blood flow; Christenson & Prior, 1978); all data were for adult ewes.

[8]Borowicz *et al.* (2005*b*). High (but subtoxic) dietary Se was fed from day 50 of gestation until necropsy.

[9]Krebs *et al.* (1997) and Parraguez *et al.* (2006) for vascularity data. Data for uterine blood flow are from humans at 36 weeks (90%) of gestation (Zamudio *et al.* 1995), as no data are available for sheep.

NSE, no significant effect.

fact, increased uterine vascular resistance and reduced uterine blood flow can be used as predictors of high-risk pregnancies and are associated with fetal growth retardation (Trudinger *et al.* 1985; North *et al.* 1994). Thus, the impact of factors that influence placental vascular development and function on fetal growth and development is striking (Reynolds & Redmer, 1995; Reynolds *et al.* 2005*a*). Moreover, observations in humans and livestock indicate that compromised fetal growth impacts not only the neonate but also health and productivity throughout life (Barker & Clark, 1997; Breier *et al.* 2001).

As summarized in Table 1, in sheep studied during late gestation, uterine or umbilical blood flows, or both, are reduced in every model of compromised pregnancy in which they have been evaluated. These models of compromised pregnancy include overfed adolescents, underfed adolescent and adult dams, as well as environmental heat stress, hypoxic stress, and multiple fetuses. These observations agree with those in women, in which placental perfusion is reduced in pregnancies with growth-restricted fetuses (Poston, 1997; Moore *et al.* 2004; Redmer *et al.* 2004*b*; Huppertz & Peeters, 2005).

In addition, although vascular development of the placenta also is decreased in several of the models of compromised pregnancy, it is increased in others (Table 1). Interestingly, in two of the models in which placental vascularity is increased (high dietary selenium or hypoxic stress; Table 1), there was no effect on fetal size, suggesting an adaptive placental response that preserves the fetal nutrient supply. In the other model exhibiting increased placental vascularity (Romanov *versus* Columbia genotype; Table 1), the animals were subject to long-term genetic selection resulting in increased litter size. This latter case resembles that of Meishan and Yorkshire pigs, in which the Meishans exhibit increased litter size and weight associated with increased placental vascularity and vascular endothelial growth factor (VEGF) expression (Biensen *et al.* 1998; Wilson *et al.* 1998; Vonnahme & Ford, 2004). Altered placental

vascular development and expression of angiogenic factors in several of the sheep models of compromised pregnancy is similar to that reported in compromised pregnancies in humans, and Mayhew *et al.* (2004) suggested that most of these changes could be 'driven' by the relative fetal hypoxia. Nevetheless, alterations in fetal growth seem to be associated with altered placental vascular development, although the functional consequences of these alterations remain to be determined (Mayhew *et al.* 2004; Huppertz & Peeters, 2005; Reynolds *et al.* 2005*a*,*b*).

Altered placental growth and vascular development has been associated with altered expression of the genes for the major angiogenic factors, including VEGF, as well endothelial nitric oxide synthase (eNOS or NOS3), which produces nitric oxide (NO) and thus is an important regulator of both angiogenesis and vasodilatation (Reynolds & Redmer, 2001; Redmer *et al.* 2005; Reynolds *et al.* 2005*a*). Placental explants from pre-eclamptic human pregnancies exhibit increased production and release of soluble VEGF receptor-1, which binds to and inhibits the activity of VEGF ligands (Ahmad & Ahmed, 2005). Thus, placental angiogenic and vasoactive factors might serve as therapeutic targets in compromised pregnancies in humans (Godfrey, 2002; Ahmad & Ahmed, 2004).

Little data are available to address whether placental expression or production of vasoactive factors other than eNOS, or placental vasoactivity itself, is altered in compromised pregnancies. Vonnahme *et al.* (2004*b*) demonstrated a 2- to 4-fold increase in the placental vasoconstrictor response to a depolarizing dose of KCl when the dams were nutrient restricted during the first 40% of pregnancy, but not when they were subsequently re-fed and evaluated late in gestation. In fact, the placental vasoconstrictor response to angiotensin II was reduced in the re-fed dams, even though expression of angiotensin receptors 1 and 2 was similar to that of the control dams (Vonnahme *et al.* 2004*a*). These responses in vasoactivity are interesting because fetal growth was reduced during the nutrient restriction, in association with the increased placental vasoconstrictor response, whereas fetal size was normal in the re-fed dams late in gestation, when the placental vasoconstrictor response was blunted. In uterine arteries of rats in which placental perfusion is reduced experimentally, the vasoconstrictor response is enhanced, whereas endothelium-dependent vasorelaxation is reduced (Anderson *et al.* 2005). Interestingly, in humans the vasoconstrictor response of placental arteries to U46619, a thromboxane mimetic, was reduced in pregnancies exhibiting pre-eclampsia or intrauterine fetal growth restriction (Wareing & Baker, 2004). Thus, although the responses are complex, placental vasoactivity seems to be altered, and vasoactive factors are therefore logical therapeutic targets in compromised pregnancies.

Potential of placental blood flow and vascularity as therapeutic targets

Fetal growth restriction, resulting in low birth weight, occurs in 7–8% of human pregnancies in the United States, and is associated with increased perinatal mortality and morbidity (NLM, 2002*a*,*b*; NVSR, 2004). Because of the importance of placental blood flow to placental function, and the recognition that placental size, utero-placental blood flows, and expression of angiogenic and vasoactive factors are reduced or altered in compromised pregnancies, it has been suggested that therapeutic agents that target placental blood flow might be used to ameliorate fetal growth restriction (Godfrey, 2002; Ahmad & Ahmed, 2004; Wu *et al.* 2004; Wareing *et al.* 2005).

As discussed in the following paragraphs, perhaps some of the best candidates are the phosphodiesterase 5 (PDE5A)-specific inhibitors, which include sildenafil, tadalafil and vardenafil (marketed under the trade names Viagra, Cialis and Levitra, respectively). These pharmacological agents enhance the vasodilatory action of NO by inhibiting the breakdown of cGMP, the second messenger for NO, thus causing sustained relaxation of vascular smooth muscle (Michel, 2006).

Nitric oxide is an important regulator of blood flow to the uterus in the non-pregnant state and also during pregnancy (Magness, 1998). Expression of both eNOS and soluble guanylate cyclase, which serves as the receptor for NO and thus mediates its effects in vascular smooth muscle, are elevated in uterine arteries during pregnancy (Itoh *et al.* 1998; Vagnoni *et al.* 1998; Zheng *et al.* 2000; Magness *et al.* 2001; Joyce *et al.* 2002). In addition, basal production of NO contributes to low feto-placental vascular resistance during pregnancy (Sladek *et al.* 1997). Circulating NO and its metabolites are elevated in pregnancies with multiple compared with single fetuses (Vonnahme *et al.* 2005). As mentioned previously, placental expression of eNOS was reduced in some models of compromised pregnancy, including various conditions associated with intrauterine growth restriction in humans (Bird *et al.* 2003; Maul *et al.* 2003; Wu *et al.* 2004; Redmer *et al.* 2005). Moreover, NO, produced by endothelial cells, and VEGF, produced primarily by vascular smooth muscle and capillary pericytes, may interact by stimulating each other's expression (Ahmed & Perkins, 2000; Reynolds & Redmer, 2001). Thus, impaired placental syntheses of NO may provide a unified explanation for fetal growth retardation in both underfed and overfed sheep models of fetal growth restriction (Wu *et al.* 2004).

Oestrogens are probably important mediators of utero-placental blood flow and vascularity changes observed during pregnancy (Magness, 1998). Oestrogen treatment of ovariectomized ewes increases uterine

blood flow and eNOS expression (Vagnoni *et al.* 1998; Rupnow *et al.* 2001). Sildenafil enhanced both basal and oestrogen-induced increases in uterine blood flow in ovariectomized ewes (Zoma *et al.* 2004). Wareing *et al.* (2005) found enhanced vasoconstrictor and reduced vasodilator responses of myometrial arteries from growth-restricted pregnancies; in the same study, sildenafil citrate significantly reduced vasoconstriction and significantly improved vasorelaxation. In a recent, and very preliminary, study (M. C. Satterfield, G. Wu & T.E. Spencer, unpublished results), we found that sildenafil administered subcutaneously from day 28 to day 112 of gestation significantly improved fetal weights in compromised pregnancies in ewes, using the maternal undernutrition model described by Vonnahme *et al.* (2003). Thus, the available data in humans and sheep support the suggestion that PDE5A inhibitors may offer a potential therapeutic tool to improve utero-placental blood flow in compromised pregnancies.

In addition, nutriceutical approaches may also be used to manipulate the NO system. Citrulline is a precursor of arginine, which is a common substrate for NO synthesis via any of the various NOS (Flynn *et al.* 2002). Fetal growth retardation induced by maternal undernutrition from day 28 to day 78 of gestation in sheep was associated with a decrease in arginine and citrulline in maternal plasma, fetal plasma, and allantoic fluid by 23–30% at day 78 of gestation (Kwon *et al.* 2004). Further, concentrations of biopterin (an indicator of *de novo* synthesis of tetrahydrobiopterin (BH_4), which is an essential co-factor for NOS) in fetal plasma, and amniotic and allantoic fluids, were reduced by 32–36% in underfed ewes (G. Wu, unpublished results), perhaps indicating reduced availability of BH_4 for NO production in the conceptus. These changes could impair placental and fetal NO synthesis, thereby resulting in reduced placental blood flow in underfed ewes (Bell & Ehrhardt, 2002; Kwon *et al.* 2004). Indeed, Xiao & Li (2005) recently reported that daily intravenous infusion of arginine for 7 days during late gestation (week 33), to women with unknown causes of fetal intrauterine growth restriction, resulted in a 6.4% increase in birth weight at term. Whether intravenous or oral administration of arginine could provide a therapeutic approach to consistently enhance fetal growth in compromised pregnancies, and whether its effects are via improved uterine and placental blood flows, are questions that, because of the simplicity of the approach, seem worthy of further investigation.

Conclusions

One of the remaining questions concerning compromised pregnancies is when in gestation are placental blood flows and angiogenesis affected and what mechanisms are responsible? In the overfed pregnant adolescent ewe, we recently found that uterine blood flow is reduced by 56% at day 90 of gestation, which is before any reduction in fetal or placental weights is observed (J. Wallace, M. Matsuzaki, J. S. Milne & R. P. Aitken, unpublished results, as cited in Wallace *et al.* 2005). This reduction in uterine blood flow corresponds with decreased expression of placental angiogenic factors, including VEGF and eNOS, by day 80 of gestation (Redmer *et al.* 2005), which is associated with altered placental vascular architecture late in pregnancy (Redmer *et al.* 2004*a*). Similarly, in heat-stressed ewes at day 55 of gestation, caruncular VEGF mRNA is unaltered but VEGF protein is reduced (Regnault *et al.* 2002*a*); for cotyledon, VEGF mRNA is elevated but protein is unaltered. These changes in angiogenic factor expression occurred before a reduction in fetal or placental weights (Regnault *et al.* 2002*a*) and were reflected by altered placental vascular architecture by day 90 (Regnault *et al.* 2002*b*). Thus, changes in placental blood flows and angiogenesis warrant further investigation in terms of their ontogeny, their regulation, and whether they occur in models of compromised pregnancy other than the overfed adolescent or heat-stressed adult.

The data we have summarized provide the basis for a convincing argument that restoration of placental blood flows and vascularity could provide an important therapeutic tool to manage compromised pregnancies for optimal fetal growth and development. However, it seems obvious that this strategy needs to be examined in much greater detail in various models of compromised pregnancy, and that it also will be important to establish whether the resulting 'rescued' fetuses or neonates are normal. It seems equally obvious that animal models of compromised pregnancy will be important in this effort.

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